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SHORT COMMUNICATION

Detection of mucormycosis caused by *Apophysomyces elegans* in a Lesser Long-nosed Bat (*Leptonycteris yerbabuenae*) in Central Mexico

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Abstract

We describe a case of mucormycosis in a Lesser Long-nosed Bat (*Leptonycteris yerbabuenae*) caused by *Apophysomyces elegans* in Puebla, Central Mexico. The diagnosis was supported by laboratory analysis and necropsy. We present the first report of the fungus in a wild host; therefore, we indicate that further studies are necessary to understand its infection cycle since this pathogen may indicate a risk of zoonotic, and anthropozoonotic diseases.

Keywords

Chiroptera, fungi, migrant species, Puebla, zoonosis

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PENSOFT.

Introduction

Black fungus (*Apophysomyces elegans*) is a saprophytic mold of the order Mucorales that subsists on decaying vegetable matter in the soil (Cooter et al. 1990; Ingram et al. 2014). It was first described in soil samples from a mango orchard in India in 1979 (Meis and Chakrabarti 2009) and has been subsequently found in soil samples from other countries. It has been detected in samples from Central New York to South Chungcheong in South Korea (GBIF.org 2021), but its distribution is not accurate (Cooter et al. 1990; Meis and Chakrabarti 2009; Ingram et al. 2014). It is an emerging pathogen in many tropical and subtropical countries (Chakrabarti et al. 2010) as it is among a group of fungi and it is the third most common cause of invasive fungal infection - IFI (Christoff et al. 2010); and its incidence in humans is increasing (Meis and Chakrabarti 2009; Chakrabarti et al. 2010; Christoff et al. 2010; Sifuentes-Osornio et al. 2012; Ingram et al. 2014). Recently, the fungus has gained importance as with the COVID-19 pandemic; infections among patients affected with or recovering from the coronavirus are on the rise (Gandra et al. 2021; Rahman et al. 2021). Inoculation has been associated with contamination of local wounds with soil or plant detritus after disrupting the skin barrier (Meis and Chakrabarti 2009; Ingram et al. 2014). Its diagnosis is difficult as the species has significant geographic variations and the clinical manifestations and subsequent mortality depend on the mode of acquisition and the host immune status (Ingram et al. 2014).

In this communication, we report a case of an infection of a Lesser long-nosed female bat (*Leptonycteris yerbabuenae*) by black fungus (*A. elegans*). This report is important because it is the first one on wild animals in Mexico and the presence of fungi in wildlife reservoirs is rare (Kruse et al. 2004). Furthermore, bats have generated different adaptations that enable them to be reservoirs of different diseases, therefore, when they get infected with certain fungi, their pathology varies from standard to extreme pathology (Brook and Dobson 2015). Also, it highlights that despite reported cases in humans increasing (Chakrabarti et al. 2003), there are only a few records of it among animals. In captivity it was present in marine mammals in the USA (Robeck and Dalton 2002) and elephants in India (Thomas et al. 2008). Although the life cycle of the species in the wild is unknown, a study on wild rats (*Rattus norvegicus*) detected it in 12.5% of the samples analyzed (whiskers, hair, tail scrapings and nails) (Thomas and Thangavel 2018). Finally, the record is of the utmost importance as despite its ample distribution the fungi has not been classified as zoonotic and transmission between animals and humans is minimal (Woolhouse and Gowtage-Sequeria 2005). Thus, this finding can support as a first step for future studies, to determine if there is any relationship between animal and human cases.

We made a systematic sample along spring (February-May of 2018) on four different places located in Puebla (Huiziltepec and Molcaxac municipalities), Central Mexico, to determinate diversity and abundance of pollinators in traditional agroecosystems of pitaya (*Selenicereus undatus*). Bats were sampled using mist nets as it is the most widespread methodology despite it can underestimate the presence or abundance of species

present (Murray et al. 1999; Faria et al. 2006). We checked the nets every 30 minutes, and all captured bats were identified, measured and sexed before they were released.

We accumulated 24 net/nights representing 2421 hours during which we captured 268 individuals. The infected individual was a Lesser long-nosed female bat (total body length: 9 cm, weight: 20g) and it was captured close to a roosting cave (18°43'22.51"N, 97°50'45.24"W). It appeared physically weak and sick. Hence the guarding veterinarian decided to sacrifice it by breaking its neck following the protocol approved by the scientific and ethic committee of the Centro de Agroecología. The individual was conserved in ice until processed.

We undertook a complete necropsy following a standard protocol (Aluja et al. 2015). We started the necropsy with a visual inspection of the anatomical plans (cranial-caudal and ventral- dorsal) to determine external injuries. Afterwards, we made an inspection starting with the head and the oral cavity to follow with the respiratory, cardiac and digestive systems. Further analysis was carried out in a certified veterinary laboratory (M and G Laboratories, Puebla). Technicians took the sample through an injury swap; the fungal study consisted of enriched culture media, selective culture media, stains, and biochemical tests. Further description of the laboratory analysis can be found in annex 1. We also made a bacterial study of the exudate through enriched and selective culture media, stains, and biochemical tests. Further analysis included a skin crapping, macroscopical and microscopical analysis.

The first examination demonstrated that the individual had several visible dermatological lesions such as dry, rounded lesions of variable size (0.4–1.5 mm) in the interdigital membranes, the posterior members, and the thoracic regions (Figure 1 (A)). The upper part of the arm presented an inflammatory zone with granulomas without anatomical changes in the wing (Figure 1 (A)). The pectoral region had a rounded alopecic zone of about 1 cm. The inferior limbs presented diffused white spots with visible granulomas and erythematous areas. The biochemical results demonstrated *A. elegans* (Figure 1 (C)) resembling the human cases (Meis and Chakrabarti 2009; Sun and Singh 2011). Besides, the individual was parasitized by mange (or scabies) mites (*Sarcoptes* spp.) which only affect individuals with a poor health condition (Pence and Ueckermann 2002). Laboratory results are important as infection symptomatology shows significant geographic variations, making it difficult to diagnose jointly with the fact that clinical manifestations and subsequent mortality depend on the mode of acquisition and the host immune status (Ingram et. al. 2014). The disease has a low prevalence as just one individual among the 268 captured after 2421 hours presented symptoms.

The necropsy demonstrated that several systems were severely affected, resembling symptoms and aggressiveness previously reported in human systems (Cooter et al. 1990; Meis and Chakrabarti 2009; Sun and Singh 2011). The respiratory system was severely affected as the thoracic cavity had pleural effusion, which has been reported in human patients (Sun and Singh 2011). The trachea and entrance of the bronchial tree had nodules, the lung parenchyma had tumors with an emphysematous aspect, and the lobules had lost their borders. Although it was not possible to

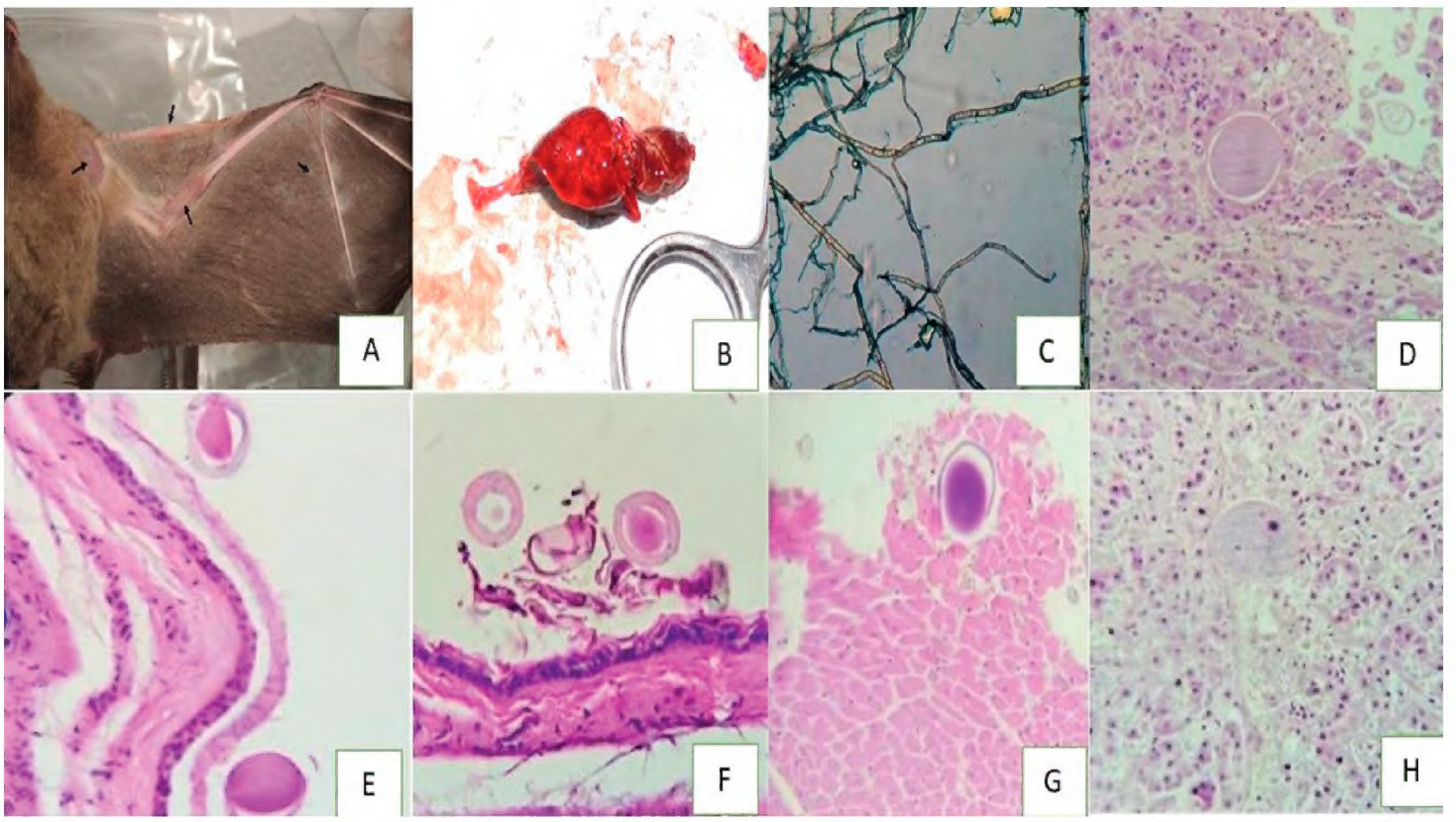

Figure 1. Lesser long-nosed female bat (*Leptonycteris yerbabuenae*) infected by black fungus (*Apophysomyces elegans)* A Skin lesions present in the bat B Changes in organ shape and coloration in lungs and heart, showing granuloma along the surface (white spots) of the lungs C *A. elegans* cultivated from the bat´s wing D–H tissues showing changes and parasitic structures.

make a detailed evaluation of the vascular system, we observed that the heart had a rounded form. Furthermore, the mitral and the tricuspid valves had a soft consistency without being friable, and the inferior apex of the myocardium had a black-purple color resembling a necrotic area. Finally, it was possible to observe that the blood vessels inside the cardiac cavity were engrossed. Changes in other organs included engrossment and a change in coloration of the small intestine, and vasculitis in the mesenteric vessels. The kidneys had a circular form, were augmented, had a soft consistency, and we could not differentiate any internal structure. Finally, in the skeletal muscle system, it was possible to find necrotic zones along the fascia and the pectoral muscles and dark areas in the psoas muscle.

These findings indicate that bats are prone to suffer murcomycosis. But it is not clear if the populations may be at risk, as just one individual presented it. However, it could be an indicator that bat populations may be at risk, especially those of *Leptonycteris yerbabuenae*, considering that the presence of *A. elegans* in a wild animal may imply that the fungus can use other mammals or soils to survive, using them as hosts or reservoirs of the pathogenic agent. This opens the possibility that the fungus may be transmitted to other individuals of the same population. The latter happens with other fungus species (e.g., *Pseudogymnoascusdestructans*) that spreads among the population if health and environmental conditions converge to allow the pathogen to establish (Verant et al. 2012; Warnecke et al. 2012). However, in the case of *A. elegans*, the conditions to develop the infection are very particular such as an individual with an inmuno depressed

system or with a chronic degenerative disease, skin trauma and in direct contact with the pathogen (Mahendra et al. 2015). Thus, it is necessary to undertake constant monitoring of bat populations as due to the ecology of the Lesser long-nosed bat (Gómez-Ruíz et al. 2015) the fungus could be easily disseminated among different populations posing a threat to the species. In addition, we do not know the real infection route and fungus preferences, thus, we emphasize the need to understand the life cycle and dynamics of *A. elegans* infection in the wild.

Our report is the first one of an animal infected with *A. elegans* in Mexico; thus, it is noteworthy that clinical manifestations resemble those reported in humans. Also, we consider that prevalence in both species is low (Chakrabarti et al. 2003), as just one of the 268 captured individuals was infected. The latter factors in developing a murcomycosis infection are particular, such as an individual with an immune-depressed system or a chronic degenerative disease, skin trauma, and direct contact with the pathogen (Pal et al. 2015). However, it is necessary to determine the infection cycle as invasive fungal infections (IFIs) have increased during the past two decades (Sifuentes-Osornio et al. 2012). Also, we emphasize that we need studies to understand the relationships between the pathogen, host, and environment to understand the effects *A. elegans* can have over bat populations.

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Supplementary material 1

Analysis results

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Data type: Pdf file.

Explanation note: Lab Analysis and result.

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